Research paper

Fennel oil and by-products seed characterization and their potential applications

Bouchra Sayed Ahmad[a,b], Thierry Talou[a], Zeinab Saad[b], Akram Hijazi[b], Muriel Cerny[a], Hussein Kanaan[c,d], Ali Chokr[c], Othmane Merah[a,*]

[a] Université Fédérale de Toulouse Midi-Pyrénées, INP-ENSIACET, Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France
[b] Doctoral School of Science and Technology, Research Platform for Environmental Science (PRASE), Lebanese University, Lebanon
[c] Laboratory of Microbiology, Department of Biology, Faculty of Sciences I, Lebanese University, Beirut, Lebanon
[d] International Center for Infectiology Research-INSERM U1111-ENS Lyon-CNRS UMR5308-Lyon, France

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ABSTRACT

The implementation of renewable resources in the industrial production processes appears to be the most effective way to achieve sustainable development. However, in order to tackle the key issues of shifting to renewable resources, a full exploitation of biomass resources and efficient utilization of complex organic macromolecules and also other chemical constituents such as antioxidants in bio-refinery system will be crucial. In this regard, fennel (*Foeniculum vulgare*) seeds could be a promising bio-resource with significant interest as a rich source of both vegetable oil (VO) and essential oil (EO), in addition to rare phytochemicals. Thus, in the present paper, a trans-disciplinary assessment of a new bio-refinery process from fennel seeds was established: the development of an integrated valorization of fennel seeds, allowing the extraction of VO and EO and their exploitation in cosmetic applications as well as the valorization of residual by-products as a source of biologically active compounds, these processes constituted the basis of this bio-refinery concept. Laboratory obtained results and pilot-scale levels with fennel seeds reported extraction of high yield of both VO and EO (19.8% and 1.8%, respectively) with significant amounts of valuable components, petroselinic acid and trans-anethole (74.8% and 70.7%, respectively). Further, the valorization of these oils as functional ingredients in moisturizing cream formulas showed a positive impact on the overall emulsions structure and quality. Next to this, fennel oilseeds by-products exhibited a remarkable antioxidant potential with high phenols and flavonoids contents and exhibited good antimicrobial properties depending on the extract type. These promising findings are of great economic interest as they can lead to a wild range of novel, bio-based industrial applications from fennel seeds.

1. Introduction

Bio refinery concept can be defined as the biomass conversion processes. It includes several conversion methods (biochemical, microbial, chemical and thermochemical) seeking for optimal use of biomass. Thus, added value chemicals, co products and residues are obtained through strategic involvement of the chemical industry in the supply of final products to different domains such as petrochemical, pharmaceutical, building, cosmetic and others (Venskutonis and Jonušaitė, 2016). Before this step, biomass and their byproducts could be evaluated for these purposes.

Fennel (*Foeniculum vulgare* Mill.) is a commercially important Apiaceae species from the Mediterranean area and central of Europe and is among the most widespread medicinal plant worldwide, being extensively grown in arid and semi arid regions as one of the oldest spice plants (Barros et al., 2010). It is recommended traditionally for gastrointestinal and neurological disorder, kidney stones, vomiting and diarrhea, it has also antispasmodic, antiseptic, carminative and anti ulcer properties (Ghanem et al., 2012). Recently much attention has been focused on fennel due to the nutritional and health protective value of their seeds that are rich in vegetable and volatile oils (Matthäus and Musazcan Özcan, 2015). Fennel seeds are considered also as source of many health beneficial compounds including minerals, vitamins, and others which explain their applications for pharmaceutical, cosmetic, perfumery and food industries (Nassar et al., 2010).

In 2014, the European Commission authorised the use of coriander oilseed as a novel food ingredient under 'Regulation (EC) No 258/97 of the European Parliament and of the Council', this is due to its richness in the uncommon monounsaturated fatty acid, the petroselinic acid (C18:1n12). This fatty acid is a positional isomer of oleic acid used as

* Corresponding author.
E-mail address: othmane.merah@iut-tlse3.fr (O. Merah).

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valuable raw material to the synthesis of a series of bio based com pounds that could be of particular interest to chemical industries (Nguyen et al., 2015). In this context, fennel oilseed could present an attractive competitor as the next novel food ingredient owing to the presence of petroselinic acid, which constitutes over 80% of all fatty acids.

Plant seeds can be processed into high quality vegetable or essential oils, the remaining portion may find various profitable applications due to their phytochemicals content and antioxidant activity. These by products can thus be seen as economically promising raw materials for future applications in industrial products for pharmaceutics or cos metics (Fekria et al., 2012).

Various vegetable oils can be applied for the moisturizing, protec tion and healing of problematic skins. Besides its nutritional benefits, fennel oil has several positive effects on the skin according to its rich ness in mono unsaturated fatty acids (MUFAs) and especially petrose linic acid which can resolve some skin problems, such as dryness (Oyedeji and Okeke, 2010). With the recent trend towards en vironmentally friendly substances and more biodegradable options and in a bio refining approach in term of valorization of fennel EO and VO in a non food applications, the use of fennel oilseed in moisturizing cream formulations seem to be a promising option. Actually, oilseeds are easily biodegradable and skin lipid compatible and thus, their using in cream formulas could reduce the use of synthetic oil such as paraffin oil (Srivastava and Sahai, 2013).

The potential nutritional and functional properties of agrowastes are studied previously such as polyphenols in hemp, flax and canola seed cakes (Teh et al., 2014), proteins, fibers and other nutrients in *Arachis hypogaea* seed cakes (Fekria et al., 2012) and antioxidant ac tivity of extracts of *Guizotia abyssinica* (Wettasinghe and Shahidi, 1999) and *Rosa damascena trigintipetala* Dieck (Abdel Hameed et al., 2012) byproducts. Regarding *F. vulgare* seeds, several quantitative estimation of protein and fiber contents and total phenols and flavonoids contents, as well as their antibacterial and radical scavenging properties have been done (Christova bagdassarian et al., 2014; Shah et al., 2015). Nevertheless, all of these studies were limited on a single aspect of investigation including oil composition or biological activity, but none have addressed all of them together. Moreover, remaining residues after oil extraction have not gotten much interest. Therefore, a new bio re fining approach was established in this study in order to fully exploit fennel seeds for a wide range of industrial applications (Fig. 1). The aim of this paper is to evaluate the chemical composition of fennel oilseeds and their potential addition to moisturizing cream formulas, we aim also to analyze the potential usefulness of byproducts fennel seeds by assessing the total phenolic and flavonoid contents, as well as the an tioxidant and antibacterial properties of their extracts.

2. Material and methods

2.1. Oil extraction and analysis

2.1.1. Essential oil extraction and analysis

Two hundred grams of sweet fennel (*Foeniculum vulgare* Mill. var. dulce) grinded seeds were immersed in 2L of distilled water contained in a 6L round bottomed flask. Distillation was carried out using a Clevenger apparatus for 180 min after boiling. The extracted essential oils were recovered and stored in a refrigerator at 4 °C.

Essential oil samples were analyzed on a Hewlett Packard 5890 series Π Gas Chromatograph coupled with a 5970 mass spectrometer and equipped with fused silica capillary columns HP 5 MS (0.25 µm × 0.25 mm × 30 m) and Carbowax (0.25 µm × 0.25 mm × 30 m). GC MS parameters with a HP 5 MS column: carrier gas: helium (flow rate: 0.6 ml/min), oven tem perature programming: rising from 60 °C to 220 °C at 3 °C/min and then held at 220 °C for 12 min. Injector temperature: 250 °C, ion source tem perature: 280 °C. Electron ionization: 70 eV; mass spectra range: 30 300 amu and 2.77 scan/s; split ratio: 1/100; injection volume: 1 µl, pentane solution. GC MS parameters with a carbowax column: carrier gas: helium (flow rate: 0.6 ml/min), oven temperature programming: at 70 °C for 2 min, rising to 220 °C at 5 °C/min and then held at 220 °C for 8 min. Injector temperature: 250 °C, ion source temperature: 280 °C. Electron io nization: 70 eV; mass spectra range: 30 300 amu and 2.77 scan/s; split ratio: 1/100; injection volume: 1 µl, pentane solution. Identification of in dividual components in the essential oils or volatile extracts was based on the comparison of their retention indices calculated with reference to a series of *n* alkanes, with those found in the literature (Adams, 2007). Fur ther identification was made by comparing their mass spectra with those in the mass spectra library of data process software (NBS75 K database, Wiley 7th NIST 98 EPA/NIH Mass Spectral Library, Mass finder 3/Hochmuth and FFNSC2/Mondello, 2nd Edition, 2011 Nov.), and also those found in pub lished data. The relative percentage of each component in the essential oil was given according to the normalization results of peaks in GC chroma tograms.

2.1.2. Vegetable oil extraction and fatty acid analysis

Vegetable oil was extracted using Soxhlet apparatus. A sample of 25 g of grounded seeds from fennel was extracted using cyclohexane as solvent, for 5 h. After extraction, the solvent was removed by rotary evaporator; the oil was kept at 4 °C in a dark bottle.

After Soxhlet extraction, VO was methylated and converted into Fatty Acids Methyl Esters (FAMEs). One milliliter of MTBE (methyl *tert* butyl ether) was added to 20 mg of VO, then; 100 µl of this solution was transferred to an insert and 50 µl of trimethylsulfonium hydroxide (TMSH) was added, the solution was gently stirred. FAMEs were ana lyzed using GC/FID. The analysis was performed using a capillary

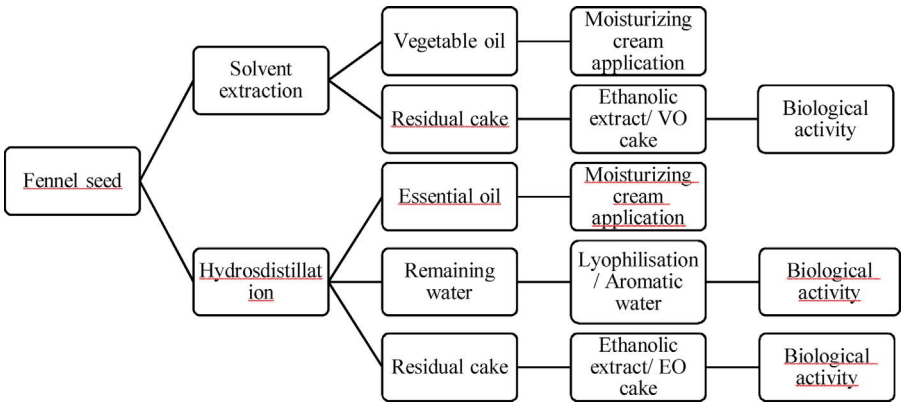

Fig. 1. Outline of bio-refining process of F. vulgare seeds applied in the present study.

Table 1
Ingredients of formulations A–C.

Phase	Ingredients (INCI name)	Content [wt.%]		
		Formulation A	Formulation B	Formulation C
Phase A (aqueous)	Aqua/water	57	57	57
	Cholorphenesin	0.2	0.2	0.2
	Carbomer	0.5	0.5	0.5
Phase B (Oil)	Cetyl alcohol	2	2	2
	Stearic acid	2	2	2
	Paraffinum liquidum	25	23.75	23.7
	Foeniculum vulgare Vegetable oil	–	1.25	1.25
	Foeniculum vulgare fruit essential oil	–	–	0.05
	Decyl oleate	7	7	7
	Ceteraeth 12	3	3	3
Phase C	Propylene gycol	0.02	0.02	0.02
Phase D	Triethanolamine	0.9	0.9	0.9
	Aqua/water	1.68	1.68	0.68
	Phenoxyethanol	0.7	0.7	0.7

column (CP Select CB for FAME fused silica WCOT i.d., 50m × 0.25 mm; film thickness, 0.25 μm). The run was under an optimized temperature program as follows: initial column temperature was 185 °C for 40 min and was programmed to increase at a rate of 15 °C/min up to a final temperature of 250 °C, and held for 10.68 min. The injector and detector temperatures were 250 °C for both. Helium was used as the carrier gas at a flow rate of 1.2 ml min^{-1} with a split ratio of 1:100.

2.1.3. Formulation of water in oil moisturizing creams

The formulations comprise oil phase, aqueous phase and other components (Table 1). Emulsions were prepared following the typical procedures used for preparing water in oil (W/O) emulsions, i.e. the oil phase was heated up to 75 °C in a glass beaker. The aqueous phase was added to the oil phase followed by homogenization with F25 Ultra turrax at 13,000 rpm for 15 min. The sample was allowed to cool down to room temperature under moderate stirring at 150 rpm, and then stored at room temperature.

2.1.3.1. Rheological measurement. Steady flow, thixotropy and viscoelastic properties were measured with a cone and plate geometry on a Modular compact Rheometer (MCR 302, Anton Paar, Austria, Europe). The cone diameter was 25 mm. Oscillatory stress sweep tests were always performed at a frequency of 1 Hz in order to know the linear viscoelasticity range. The shear rates were from 1 to 100 s^{-1} in steady flow and thixotropy property measurements, rheology curves were generated by using viscosity vs. shear rate. The measuring temperature was 25 °C.

2.1.3.2. Determination of peroxide value. Weighed 4 g of sample in 250 ml flask, and added 30 ml of acetic acid and chloroform solution (3 V/2 V) and swirl it to dissolve. 0.5 ml of KI solution was added with continuous shaking and 30 ml of water ID also added. Then titrate it with 0.01N sodium thiosulfate solution with vigorous shaking until yellow is almost gone. Add 0.5 ml of 1% starch solution and continue titration with vigorous shaking to release all I_2 from chloroform layer, until blue color disappears (Sapino et al., 2005). A control test was carried out under the same conditions (V_0 of sodium thiosulfate 0.01N), formulation A was used for V_0 determination.

$PV = 10\ (V_1 - V_0)/P$ expressed as meq/kg

P: exact mass of weighted formulation (g)

V_1: volume (ml) of sodium thiosulfate 0.01N used for the test

V_0: volume (ml) of sodium thiosulfate 0.01N used for the control test

2.1.3.3. Sensory evaluation. Twenty female panelists (ages 23 30) completed a special questionnaire concerning 4 parameters. Assessors were first introduced to the general concept of the study, following a detailed explanation of the test and used sensory descriptors. Each parameter was rated on a category scale with predefined descriptive terms. Each sample was assigned a random three digit code and presented in similar containers. The panelists received the cream samples and an analysis form, containing instructions to compare the formulations by indicating the descriptive term that better described the sensory attribute under evaluation. Each product (about 2 mg) was applied over the back of the left hand.

2.2. Biological and chemical analyses of by products

Residual oilseed meals from VO or EO extraction were subsequently extracted by Soxhlet apparatus using ethanol as solvent; the obtained extracts in addition to the water remaining from the hydro distillation were lyophilized.

2.2.1. Determination of total phenol content (TPC)

Total phenolic content was determined using the modified Folin Ciolcalteu method (Slinkard and Singleton, 1977). The absorbance was read at wavelength 765 nm. All measurements were done in triplicate for each extract. The amount of total phenol was calculated as a gallic acid equivalent (GAE) from the calibration curve of gallic acid standard solutions with a concentration range between 50 and 500 mg/L. The total phenolic content was expressed as milligrams of GAE per gram of extract (mg GAE/g extract).

2.2.2. Determination of total flavonoid content (TFC)

The total flavonoid content of seed cakes and residual water was determined using the aluminium chloride assay through colorimetry (Samatha et al., 2012). The absorbance of the reaction mixture was measured at 510 nm with a UV Visible spectrophotometer after 15 min of incubation. Distilled water was used as blank. Quantification was done based on a standard curve of rutin and expressed in mg of rutin equivalents per gram of extract (mg Ru/g extract).

2.2.3. Determination trolox equivalent antioxidant capacity (TEAC)

Radical scavenging activity against stable DPPH radical was determined by a modified method of Brand Williams et al. (1995) (Brand Williams et al., 1995). The absorption was read at 515 nm on the UV vis spectrophotometer. Each assay was repeated three times and measurement was carried out at room temperature in 30 min. The methanol solutions of Trolox with known concentrations ranging from 100 to 750 μmol/L were used for calibration.

2.2.4. Determination of antibacterial activity

2.2.4.1. Bacteria strains. Three Gram positive bacteria [*Staphylococcus epidermidis* CIP 444, *S. aureus* ATCC 25923 and *Enterococcus feacalis* ATCC 29212] and two Gram negative strains [*Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853] were used in this study.

2.2.4.2. MIC and MBC assays. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined using a microtiter broth dilution method (Sebaaly et al., 2014).

Serial two fold dilutions of different extracts in MHB (Mueller Hinton Broth) were prepared in a 96 well plate (200 µl Per Well) (Corning® Costar® 3598; Corning, NW 14831, USA). Wells with no extract added were used as a positive growth control. A diluted bacterial suspension was prepared from each strain and added to each well to give a final concentration of 5×10^5 Colony Forming Units (Cfu)/ml, Confirmed by viable counts. Wells without bacterial inoculum were used as a negative growth control. The plates were incubated for 24 h at 37 °C. The contents of the wells showing no visible growth were plated on brain heart agar (BHA) and the number of colonies was counted after overnight incubation at 37 °C to determine the MBC.

The MBC was defined as the lowest concentration reducing the initial inoculum by ≥99.9%. The MIC and MBC were determined for all strains. For each strain, at least three independent determinations were done and the modal value was taken.

2.3. Statistical analysis

All experiments were performed in triplicate and the results were presented as the mean ± SD. One way ANOVA and Tukey test by pairewise at 5% probability level were used for the analyses. The linear correlation coefficients R^2 were calculated using Microsoft Excel 2010 software.

3. Results and discussion

3.1. Essential oil yield and chemical composition

Foeniculum vulgare is widely grown in Canada, Europe, Asia and Mediterranean countries as volatile oil producing crop. Essential oil of fennel seeds was isolated from our sample with a yield of 1.84 ± 0.04% (w/w on the dry weight basis). Essential oil constituents, retention index, retention time and relative percentage of fennel seeds are summarized in Table 2. Chromatographic analysis revealed the occurrence of sixteen volatile compounds representing 99.7% of the total amount of extracted essential oil. The main volatile active compounds present in fennel essential oil were *trans* anethole (70.7%), and fenchone followed by anisketone and *p* anisaldehyde. Essential oil yield and quality are dependent on many factors such as genetic and environmental conditions as well as sampling (Barragan Ferrer et al., 2016; Roche et al., 2010). However, in this study, examined *F. vulgare* seeds appeared to be moderately rich in volatile oil compared to previous studies where essential oil contents have been reported to range between 0.69 and 4.6%. Regarding volatile oil ingredients, the identified compounds in this study were in line with the literature data which showed that the main component of sweet fennel is *trans* anethole (up to 80%) while the fenchone does not exceed 7.5% (Hammouda et al., 2014; Rahimi and Ardekani, 2013). Results obtained in these previous studies have been achieved on different culture conditions but also with genetic backgrounds different from the cultivar studied in our work. Thereby, we may suggest, according to all these studies that trans anethole and fenchone are a significant marker of essential oil compounds of fennel seeds since they have been identified in all of these studies whatever are the seeds origins, cultivars or the methods of extraction.

Table 2
Essential oil and Fatty acid composition of *F. vulgare* seeds.

No.	Volatile Compounds	RI	RT	%
	Monoterpene Hydrocarbons			
1	α-Pinene	933	6.87	**0.20**
2	Sabinene	973	8.15	**0.05**
3	β-Pinene	977	8.27	**0.63**
4	β-Myrcene	991	8.73	**0.63**
5	*p*-Cymene	1024	10.01	**1.07**
6	D-Limonene	1028	10.16	**3.28**
8	γ-Terpinene	1058	11.36	**0.34**
	Oxygenated monoterpenes			
9	Fenchone	1088	12.59	**5.72**
10	Camphor	1144	14.97	**0.13**
12	Cuminic aldehyde	1239	19.17	**2.89**
13	Carvone	1244	19.36	**2.66**
	Phenylpropanoids			
11	Estragol	1158	17.37	**3.65**
15	Trans-anethole	1291	21.43	**70.72**
	Others			
7	Eucalyptol	1031	10.27	**0.03**
14	p-Anisaldehyde	1254	19.81	**3.79**
16	Anisketone	1379	25.23	**4.07**
	Monoterpene Hydrocarbons		6.20	
	Oxygenated monoterpenes		11.40	
	Phenylpropanoids		74.37	
	Others		7.89	
	Fatty Acid		**%**	
1	Palmitic acid (C16:0)		**5.34**	
2	Stearic acid (C18:0)		**1.17**	
3	Petroselinic acid (C18:1n12)		**74.80**	
4	Oleic acid (C18:1n9)		**4.74**	
5	C18:1nc		**0.46**	
6	Linoleic acid (C18:2n6)		**12.74**	
7	Arachidic acid (C20:0)		**0.34**	
8	Linolenic acid (C18:3n3)		**0.37**	
	SFA		6.85	
	MUFA		80.00	
	PUFA		13.11	
	PUFA/SFA		1.91	

RI: Retention index relative to standard mixture of *n*-alkanes.
RT: Retention time (min).
SFA: Saturated Fatty Acids.
MUFA: Mono-unsaturated Fatty Acids.
PUFSA: Poly-unsaturated Fatty Acids.
Bold value signifies the part of each component from a total of 100%.

3.2. Vegetable oil content and fatty acid composition

Vegetable oil content in our fennel seeds was obtained 19.80 ± 0.5% (w/w on the dry weight basis). The results of fatty acid composition expressed as the mean percentage value of each fatty acid with respect to the total amount of fatty acids are shown in Table 2. Results showed that fennel vegetable oil was mainly a source of petroselinic acid followed by linoleic acid, whereas the levels of other compounds were present with lower concentrations (Table 2). Polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) index express the relationship between saturated and polyunsaturated fatty acids content in vegetable oils. It is considered as important parameter for determination of oil nutrition value, PUFA/SFA index higher than 1 reveals an oil with high nutritional value (Lawton et al., 2000). The result of our investigation showed a PUFA/SFA index higher than 1, thus fennel oil can be considered as oil with high nutritional value.

Our overall results regarding oil content and composition are within the range of prior studies where oil content ranged from 12.2 to 22.8% and the amount of petroselinic acid up was to 80% (Ali et al., 2016; Matthäus and Musazcan Özcan, 2015). However, many studies emphasized the effect of genetic characteristics and agronomic traits on vegetable oil yield and composition (Beyer et al., 2015; Hemingway et al., 2015). On the other hand, our results prove also that fennel seed oil is no less important than coriander seed oil in terms of yield and

Table 3
Relative storage modulus, loss tangent and peroxide values of formulations A–C.

	G'- End point of the linear viscoelastic region [Pa]	Tan δ	Peroxide values (meq/kg)
Formulation A	1445.1[a] ± 15.1	0.317[b] ± 0.005	Control
Formulation B	1117.2[b] ± 14.1	0.365[a] ± 0.006	2.27[a] ± 0.07
Formulation C	1123.9[b] ± 15.3	0.369[a] ± 0.007	1.94[b] ± 0.01

[a] [c]Mean values followed by different superscripts in a column are significantly different.

composition as oil yield obtained in previous studies did not exceed 25.1% with 78.2% of petroselinic acid (Nguyen et al., 2015; Sriti et al., 2011; Uitterhaegen et al., 2016).

3.3. Rheological properties of moisturizing creams enriched with fennel oil

As can be seen in the results of Table 3, formulations B and C, which contains vegetable and essential oils from fennel seed, were less vis coelastic than formulation A (control cream). The values of storage modulus (G') indicated a great difference in elasticity between cream with added oils and control cream (Table 3). The most elastic structure can be seen from the greatest G' values in the oscillation sweep test, creams with fennel oils were less elastic than the control cream.

The less elastic behavior of formulations with fennel oils in com parison with control cream is also supported by the loss tangent (tan δ) obtained values. However, tan δ is the ration of loss modulus (G") and storage modulus (G'), the higher the loss tangent is, the less elastic is the material; tan δ of control cream was lower than formulations B and C. Moreover, values of tan δ can be used for cream classification: values of tan $\sigma < 1$ indicate an elastic behavior while values of tan $\delta > 1$ mean a viscous behavior. Thus, based on our tan δ values, all of our cream formulations can be classified as elastic. On the other hand, G′ (storage modulus) describes the elastic behavior of a sample and G″ (loss modulus) represents the viscous portion, in the present study. G′ is above G″ in the linear viscoelastic range in all formulations (LVE range, Fig. 2a), which indicate also an elastic behavior. So, the solid like (elastic) property dominates over liquid like (viscous) property in our formulations, it means that all of our samples display solid like property in storage indicating that the sample will only start to flow when in fluenced by additional external forces and thus a good product stability (Montenegro et al., 2015).

Cream structure is a key parameter to determine its behavior while skin application. In the present study, the addition of fennel oil show no significant effect on cream structure, all of our formulations displayed a pseudoelastic behavior also known as shear thinning behavior, as the viscosity declined with increasing of shear stress. This behavior is ty pical of many commercial systems since it improves spreading and penetrability of the product on the skin after topical preparations (Zhang and Liu, 2013) (Fig. 2b).

Regression curves of all of our formulations show that their fluidity is restored shortly after shear stress removal, this thixotropic behavior indicate that all formulations can show an acceptable spreadility during skin application (Moravkova and Filip, 2014) (Fig. 2c).

3.4. Peroxide index of moisturizing creams enriched with fennel oil

The peroxide index is one of the most common parameter used to characterize oxidative rancidity. It is a measure of the concentration of peroxides and hydro peroxides formed in the initial stages of lipid oxidation (Sayyari and Farahmandfar, 2017). A product with peroxide

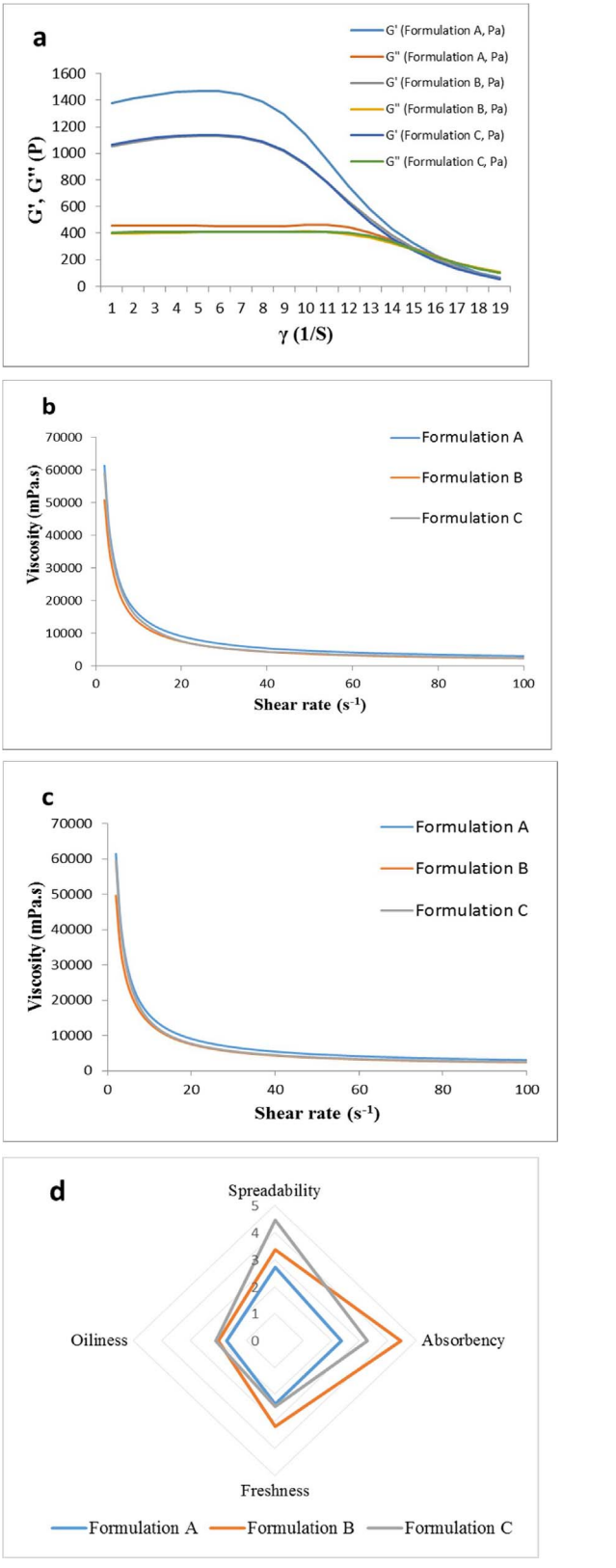

Fig. 2. Storage modulus (G') and loss modulus (G") vs shear rate (γ) of formulations A–C (a) Flow curves of formulations A, B and C in the range of shear rate 1–100 S ^{1} (b) Regression curves of formulations A, B and C in the range of shear rate 1–100 S ^{1} (c) Sensory evaluation of formulations A–C during the application on the skin (d).

value between 1 and 5 meq/kg is classified at low oxidation state; that between 5 and 10 meq/kg at moderate oxidation and above 10 meq/kg is classified at high oxidation. Nevertheless, the number of peroxides existing in a product reveals its oxidative state and hence its tendency to turn into rancid. Unsaturated fatty acids easily react with oxygen to form peroxides. Therefore, it is crucial to determine the peroxide value of our formulations after oil addition as fennel vegetable oil is con sidered as a rich source of unsaturated fatty acid, formulation A with non added oil was used as control.

Obtained peroxide values were relatively below the maximum limits, revealing a highly stable formulation against oxidation. Peroxide value of formulation B was statically higher than formulation C (Table 3), this decreasing in peroxide value was expected as fennel essential oil is rich in antioxidants which can react with radicals and thus prevent peroxide formation (Chang et al., 2013).

3.5. Sensory profile of moisturizing creams enriched with fennel oil

The appropriate sensory features constitute the most significant part of a product's sales potential and thus, the product's failure could be attributed to a gap between its sensory characteristics and customers' requirements and expectations. The results of the sensory analysis (Fig. 2d) confirmed the positive impact of fennel oils on the cream sensory characteristics (Moravkova and Filip, 2014). Formulation C received more positive assessment than formulations A and B for the spreadability. Different distribution occurs regarding freshness and absorbency characteristics, where creams B was evaluated as more fresh and can be more absorbed while skin application, followed by cream C and then cream A. The assessment of oiliness on the skin gives similar values for all formulations (Fig. 2d).

3.6. Total phenolic content (TPC) and total flavonoid content (TFC) of fennel by products

Phenols and flavonoids constitute one of the major groups of com pounds acting as antioxidants and having different therapeutic and protective effects on human health. It has been reported that agro in dustrial by products can be used as a source of phenolic and flavonoids compounds as well as a good source of natural antioxidants(Teixeira et al., 2014). Therefore, total phenol and total flavonoid contents of seed cakes after vegetable and essential oils extraction and residual water of hydro distillation from *F. vulgare* seeds were evaluated. As shown in Table 4, the phenol and flavonoid contents of our samples vary depending on the origin of the seed and the type of residue. In general, aromatic water showed the highest total phenolic content while the residual meal of the hydrodistillation had the lowest content. A similar trend is observed in the case of flavonoid content. This may be due to the dissociation of the phenolic compounds in the aromatic water, thus a low content remain in the residual meal. These results are in line with those obtained by Chatterjee et al. (2012), who found that the aqueous extract of fennel seeds contains the highest amount of phenols (Chatterjee et al., 2012). On the other hand, contents variation between different origins can be attributed to several factors such as stage of seed maturation, climate and culture conditions. Mariangela et al. (2008) found a significant difference in the total content of phe nols and flavonoids in fennel seeds from different Mediterranean countries (Mariangela et al., 2008).

Table 4
Total phenolic content (TPC), total flavonoid content (TFC) and trolox equivalent antioxidant capacity (TEAC) and minimum inhibitory concentration (MIC) and minimum bactericidal activity (MBC) of on gram- positive and gram negative bacterial strains of fennel seed by-products.

		VO Cake	EO Cake	Aromatic Water
TPC (mg GAE/g extract)		25.13^{b} ± 0.04	15.74^{c} ± 0.08	32.61^{a} ± 0.01
TFC (mg Ru/g extract)		13.09^{b} ± 0.04	5.22 ^{c} ± 0.01	18.59^{a} ± 0.11
TEAC (TE µmol/g extract)		35.85^{b} ± 0.16	26.38 ^{c} ± 0.24	60.36^{a} ± 1.05
MIC (mg/mL)	*S. aureus*	0.357	0.166	0.130
	E. faecalis	0.178	0.332	> 0.130
	S. epidermidis	0.089	0.166	> 0.130
	E. coli	0.178	0.166	0.130
	P. aeruginosa	0.357	> 0.332	> 0.130
MBC (mg/mL)	*S. aureus*	0.357	> 0.166	0.130
	E. faecalis	0.178	0.332	> 0.130
	S. epidermidis	0.178	> 0.332	> 0.130
	E. coli	> 0.357	0.166	0.130
	P. aeruginosa	> 0.357	> 0.332	> 0.130

[a] [c]Mean values followed by different superscripts in a raw are significantly different.
VO cake: residual cake after vegetable oil extraction.
EO cake: residual cake after essential oil extraction.

3.7. Trolox equivalent antioxidant capacity (TEAC) of fennel by products

The seed cakes after vegetable and essential oils extraction and the residual water of hydro distillation were investigated also for their antioxidant activities by evaluating Trolox Equivalent Antioxidant Capacity (TEAC) assay. Extracts from seed cakes showed different free radical scavenging capacities. Results showed that aromatic water re sidual from EO extraction has the highest TEAC value followed by VO cake and EO cake (Table 4). A strong antioxidant activity exhibited by fennel seeds was reported by several previous studies (Esmaeilzadeh Kenari et al., 2014; Patel and Jasrai, 2015). Such differential scaven ging activities can be due to the presence of different types of bioactive compounds especially phenolics in the extracts, to the extraction method but also to the type of used solvent. Gallic acid, caffeic acid, ellagic acid, quercetin and kaempferol are the main phenolic com pounds identified in fennel seed extracts (Li et al., 2015; Dua et al., 2013).

A linear and positive correlations were observed between phenol and flavonoid contents (R^2 = 0.99) as well as phenol content and an tioxidant activity (R^2 = 0.82). Thus, extracts with high total phenolic and flavonoids content exhibited relatively high antioxidant activity which supports the hypothesis that phenolics and flavonoids contribute significantly to the DPPH radical scavenging capacity of extracts. Such a good correlation was reported by previous studies on several species including *Pimpinella barbata* and *Coriandrum sativum* and others (Almeida et al., 2011; Christova bagdassarian et al., 2014; Javanmardi et al., 2003; Namjooyan et al., 2007).

3.8. Antibacterial activity of fennel by products

Antibacterial activity of different extracts against Gram positive and Gram negative strains using microtiter broth dilution method is pre sented in Table 4. The results of antibacterial activity of VO cakes showed high inhibition against *S. epidermidis*, less inhibition was asso ciated with *S. aureus* and *E. feacalis* and no bactericidal activity was detected against Gram negative strains (*E. coli* and *P. aeruginosa*). EO cakes showed moderate inhibitory and bactericidal activities against all strains excluding *P. aeruginosa* were no inhibition or bactericidal ac tivity was observed. Residual water showed high activity against *S. aureus* and *E. coli*, however, it did not exhibit neither inhibitory nor bactericidal activity since its MIC and MBC were out of range (Table 4). Among all extracts, the highest bactericidal activity was achieved by residual water against *S. aureus* as its MBC value was the lowest. These findings are in agreement with previous studies done by various authors (Manonmani and Khadir, 2011; Singh and Singh, 2000) who reported that aqueous extract of fennel seed was the most active among other extracts. This differential antibacterial activity can be related firstly to the characteristics of each bacterial strain, and secondly to the presence of different phytochemicals such as phenols, flavonoids, tannins, alka loids and others in the seeds residues (Sudhira et al., 2015).

4. Conclusion

The goal of this paper comprises an overall evaluating of the po tential of *F. vulgare* seed as a feasible renewable resource in a bio re finery approach. A key element of measuring the importance of fennel in the industrial process lies in characterization their oilseeds. This study revealed that oilseeds could be a novel source of highly valuable molecules including petroselinic acid and trans anethole. These oils exhibit also a positive impact on moisturizing cream formulas without altering their rheological properties. Fennel seed by products retain a good phenolics and flavonoids contents as well as good antioxidant and antibacterial activities. In addition, aromatic water has significantly higher levels of TPC, TFC and antioxidant activity while VO cake shows the best antibacterial activity. Previous studies have been conducted on coriander seeds using extrusion for sequential extraction that could be developed on this species also.

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